Reproductive and Sexual Maturity of the Mediterranean Fruit Fly, *Ceratitis capitata* (Wiedemann)[1,2]

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ABSTRACT

The data presented suggest temporal differences between reproductive maturity and sexual maturity in males as well as females of *Ceratitis capitata*. Males were reproductively mature near the time they eclose though they were not sexually mature until 48 h later. Females, on the other hand, were sexually mature at 48 h but were not reproductively mature until 4 days old. Thus, in comparision, sexual maturity in both sexes was reached at about the same time while reproductive maturity differed by about 4 days.

INTRODUCTION

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) family Tephritidae, is one of 3 fruit fly pests in Hawaii. Together with the other 2 tephritid species, *Dacus cucurbitae* Coquillett and *D. dorsalis* Hendel, the Mediterranean fruit fly has restricted the growth of the fruit and vegetable industry through crop damage, treatment costs and export limitations. Therefore, basic research is essential for the understanding of aspects on their life history strategies, physiology and behavior which may be important in the implementation, advancement and/or the develpment of control programs.

In referring to the Mediterranean fruit fly, various mating behavior terms such as sexual development (Wong and Nakahara, 1978), mating response (Wong and Nakahara, 1978) and reproductive instinct (Feron, 1962) have been used. Yet, although each term essentially conveys the same meaning, the use of these 3 terms to describe the same or similar action has been confusing. These terms have also been used to express the ability of these flies to perform those behavioral actions in courtship leading to copulation and has led to the further confusion of their definitions. The use of these terms also carries the implication that mature eggs and sperm are present at the time when copulation occurs. Thus, for the sake of clarity, the following terms will be described: reproductive maturity and sexual maturity. Reproductive maturity will refer to the presence of mature eggs and sperm. Sexual maturity will refer to the ability to perform and to receive the proper courtship stimuli leading to copulation. Data are presented to show the earliest age when each type of maturity is present in males and in females. Such data provide important information when conducting mating experiments in that only sexually mature individuals are used regardless of the reproductive state of the flies.

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MATERIALS AND METHODS

Rearing and Preparation of Flies

Laboratory reared Mediterranean fruit flies were obtained as puparia from the mass reared culture (Tanaka et al., 1970) of SEA-AR (Science Education Administration-Agriculture Research. The flies used in the sexual maturity tests were placed as puparia into containers and within 24 h of eclosion, the adults were sexed while immobilized with cold air (0 °C). The sexed flies were then placed into separate containers and provided with food (Tanaka et al., 1970) and water.

The flies used in the reproductive maturity tests were sexed immediately after eclosion and held in separate cages for determined lengths of time until they were dissected. The females used in the reproductive maturity tests were laboratory reared at 21 °C. The males used in the reproductive maturity tests were reared from different substrates or under different conditions. The following types of males were used in the reproductive maturity tests: males laboratory reared at 21 °C during pupal development, males laboratory reared at 27 °C during pupal development, males laboratory reared at 21 °C during pupal development that 1 day prior to eclosion were irradiated with a dosage of 14,000 rads from a cobalt-60 source, and males reared at 21 °C from Jerusalem cherries, *Solanum pseudocapsicum* L. collected from Kipuka Ki, Volcanoes National Park, Hawaii. All the flies used in the reproductive maturity experiments were provided with food (Tanaka et al., 1970) and water prior to their use.

Maturity Tests

Male reproductive maturity was determined by examining the testes for the presence of motile sperm. At selected time intervals, males were dissected and the testes removed from the abdomen. The testes were placed onto a microscope slide in a drop of saline solution. An incision was made near the basal end (nearest the vas deferens) of the testes releasing the contents. Sperm motility was assayed under 250X magnification by scanning the contents of the testes. Reproductive maturity was determined for males laboratory reared at 21 °C and 27 °C, males laboratory reared at 21 °C that were irradiated 1 day prior to eclosion at a dosage of 14,000 rads, and males reared from Jerusalem cherry fruits.

Female reproductive maturity was determined by examing the ovaries for the presence of mature eggs at selected time intervals. The ovaries were dissected out and then examined under 120X magnification.

To determine sexual maturity in males, 25 unmated laboratory males less than 24 h from eclosion were placed into a cage with 25 unmated 7-day-old laboratory reared females. Previous determination that females were receptive at 7-days-old was made, thus, this combination determined the earliest age that males would mate.

The same procedure which was used to determine male sexual maturity was used to determine female sexual maturity with the ages of the sexes reversed. As a control, 25 unmated 7-day-old females were placed into a cage with 25 unmated 7-day-old males during the same experimental period. Also, 25 males and 25 females were placed into a cage at ages less than 24 h from eclosion. In all cages, the number of matings and the age of the flies when matings occurred were recorded. The observations were made daily for a period of 4 days between

0800 and 1200 h. Wong and Nakahara (1978) as well as personal observations indicated that this time interval contained the peak hours of sexual activity.

RESULTS AND DISCUSSION

Reproduction Maturity and Sexual Maturity in Males

The laboratory males reared at 21 °C and 27 °C were found to be reproductively mature at about 1 h after eclosion with 93.3% and 96.7%, respectively, of the males having motile sperm at this age (Table 1). The presence of motile sperm near the time of eclosion indicates that though the duration of the pupal development decreased with increasing temperature, spermatogenesis appears to be synchronized with other physiological features needed for development. The percentage of laboratory males with motile sperm increased with age. One hundred % of the wild males that were reared from field fruits had motile sperm at eclosion (Table 1). Thus, in comparison, there does not appear to be any temporal difference in reproductive maturity between wild males and laboratory reared males.

About 95% of the irradiated laboratory males dissected within 1 h of eclosion had motile sperm in their testes. Further dissections of irradiated laboratory males 24 h later showed that 96.7% of the males were reproductively mature. Therefore in comparing the percentage of reproductive maturity in males between untreated laboratory reared and irradiated males, it is clear that irradiation does not affect reproductive maturity. However, the effects of irradiation on the viability of the sperm is another facet which is not considered here. Also, the increase in the percentage of irradiated males with motile sperm with age is an indication that the radiation process does not delay spermiogenesis.

The data presented in Table 2 indicates that males require a maturation period of at least 48 h from eclosion before mating occurs. This indicates that male reproductive maturity and sexual maturity are developing at different rates or times. Thus, although mature spermatozoa may be present near the time of

TABLE 1. Reproductive Maturity in Males as Determined by the Presence of Motile Sperm in the Testes.

Type of Male Dissected	Age (h)	# of ♂♂ with motile sperm	# of ♂♂ dissected	% of ♂♂ with motile sperm
Laboratory reared at 21 °C	0-1	42	45	93.3
Laboratory reared at 21 °C	8-10	6	6	100.0
Laboratory reared at 21 °C	13-15	5	5	100.0
Laboratory reared at 21 °C	19-20	4	4	100.0
Laboratory reared at 21 °C	48-49	30	30	100.0
Laboratory reared at 21 °C	72-73	25	25	100.0
Laboratory reared at 27 °C	0-1	29	30	96.7
Irradiated, reared at 21 °C	0-1	43	45	95.6
Irradiated, reared at 21 °C	24-25	29	30	96.7
Wild ♂♂ reared from Jerusalem cherries	0-1	30	30	100.0

eclosion, males appear to need some time after reproductive maturity before mating can be achieved. However, Kobayashi (pers. comm.) indicates that with another technique (Anwar et al., 1970), the earliest age when mature sperm are observed in the testes is 24 h. But, though a temporal difference of up to 24 h may exist between the two techniques, the order of achieving reproductive maturity and sexual maturity is not altered.

Reproductive Maturity and Sexual Maturity in Females

In laboratory females, reproductive maturity is not reached until at least 4 days after eclosion (Table 3). But, in comparison to reproductive maturity, females may be receptive to the courtship displays of males as early as 2 days following eclosion. The observation that females are sexually mature prior to reproductive maturity indicates that the transferred sperm which is stored in the spermatheca is used for later use.

The ability of females to mate prior to reproductive maturity has genetic and biological significance. First, the insemination of the females prior to ovarian maturity insures that there will be no gametic wastage since all the eggs oviposited are all likely to be fertilized. Secondly, the sexual receptivity of females prior to ovarian development and the delay in males to mate even after reaching reproductive maturity appears to increase the probability for inbreeding. However, when newly emerged males and females were placed into the same cage, mating did not occur until the flies were 3 days old (Table 2), a day later than newly eclosed females placed with older males. Not only was mating delayed but the overall frequency of mating when newly eclosed females were placed with newly eclosed males was also lower. Thus, given the same situation in nature, newly emerged females would be more likely to mate with older males than her brothers that emerged at the same time.

TABLE 2. Sexual Maturity of Males and Females as Determined by Mating Between Combinations of 7-day-old and 24-hours-old Flies.

Age of ♀♀ and ♂♂	# of Matings on Experimental Day				Total # of Matings
	1	2	3	4	
24-h ♀♀ with 24-h ♂♂	0	0	2	5	7
24-h ♀♀ with 7-day ♂♂	0	1	12	16	29
7-day ♀♀ with 24-h ♂♂	0	3	20	12	35
7-day ♀♀ with 7-day ♂♂	25	10	1	2	38

TABLE 3. Reproduction Maturity in Females as Determined by the Presence of Mature Eggs in the Ovaries.

Age (h)	# of ♀♀ with mature eggs	# of ♀♀ dissected	% of ♀♀ with mature eggs
0-24	0	15	0
24-48	0	15	0
48-72	0	15	0
72-96	1	30	3.3
96-120	4	30	13.3
120-144	31	43	72.1
144-168	30	36	83.3
168-192	30	30	100.0

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